Preparation of B-Lymphocyte Lysates for Cyclic AMP Determination

AfCS Procedure Protocol ID PP0000001200
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The method chosen for measuring the content of cyclic adenosine 3',5'-monophosphate (cyclic AMP or cAMP) in splenic B lymphocytes (B cells) is an enzyme-linked immunoassay system developed by Amersham Biosciences. An overview of the assay, cAMP Biotrak EIA, and its instructions, Biotrak Protocol, are available through the indicated links. The procedure described below provides a sufficient sample for several determinations of cAMP using the acetylation protocol.

Treatment of Cells and Preparation of Extracts

1. Suspend freshly isolated splenic B cells at 16.7 x 10^6 cells/ml in Supplemented Iscove's Modified Dulbecco's Medium (SIMDM) and distribute 0.090 ml into individual wells of 96-well tissue culture plates as needed.
2. Incubate at 37 °C in air and 5% CO_2 for 1 hr.
3. Transfer the plate to an environmental chamber containing air at 37 °C.
4. At timed intervals, add 0.010 ml of ligand working stock (10X final concentration in SIMDM) or vehicle control working stock (in SIMDM) to appropriate wells to begin treatments. (Note: vehicle controls constitute matching dilutions of solvents in which ligands are dissolved and stored.)
5. Mix with a microtiter plate shaker for 2 sec at 350 rpm.
6. Incubate at 37 °C in the environmental chamber.
7. At the desired times, end treatments with the addition of 186 µl of 100% ethanol and mix by pipetting up and down 3 times. (A multichannel pipette is used for parallel time courses with several ligands.)
8. After all of the samples on the plate have been stopped, allow lysates to sit on ice for 5 min. Centrifuge the plate at 2000 x g for 15 min at 4°C. Transfer the lysates to barcoded, 0.6-ml Eppendorf tubes.
9. Add 100 µl of 65% ethanol to the same wells and mix by tilting the plate in a circular motion to rinse the wells. Centrifuge the plate at 2000 x g for 5 min at 4°C.
10. Combine the supernatant (step 9) with the appropriate lysates (step 8) in the 0.6 ml Eppendorf tubes.
11. Dry the samples in a vacuum with centrifugation and heat (Speed Vac) until the samples are completely dry (about 2 to 3 hr).
12. Secure caps on the samples and store at –80 °C until determination of cAMP by enzyme immunoassay (EIA).

Reagents and Materials

Supplemented Iscove's Modified Dulbecco's Medium (SIMDM): AfCS Solution Protocol ID PS0000005600

96-well, tissue culture plates: Falcon; catalog no. 353072

Ethanol, 100%: Aaper Alcohol and Chemical Co.; catalog no. 030801

Eppendorf tubes, 0.6 ml: Sarstedt; catalog no. 72.699

Ethanol, 65%: AfCS Solution Protocol ID PS0000007500

Speed Vac System: Savant; catalog no. SS21

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